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Original Article

COMPARISON OF SHORT-TERM *IN VITRO* FLUORIDE RELEASE AND RECHARGE FROM FOUR DIFFERENT TYPES OF PIT-AND-FISSURE SEALANTS

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Abstract

Objective: The purpose of this *in vitro* study was to assess the effects of four commercial fluoride-containing pit-and-fissure sealants on caries prevention. *Materials and Methods*: Four sealants containing fluoride, Fuji III, Fuji III LC (GC Co., Tokyo), Teethmate F-1 (Kuraray Medical Co., Osaka) and Helioseal F (Vivadent Co., Liechtenstein) were used to investigate fluoride release and recharge. Disk-shaped specimens prepared from each material were immersed in distilled water at a temperature of 37°C. After seven days, acidulated phosphate fluoride solution (APF) was applied to each specimen, and it was then again immersed in distilled water for 14 days. We then determined how much fluoride had been released into the immersing water. Fuji III LC was used with APF solution to investigate the fluoride uptake. *Results*: Fuji III had the highest fluoride release, and Fuji III LC had the highest fluoride recharge. Helioseal F and Teethmate F-1 had almost no fluoride recharge. Fuji III LC/APF had a higher fluoride uptake to enamel than Fuji III LC. *Conclusions*: These results suggest that GIC-sealants in the oral cavity can serve as a fluoride reservoir and contribute to retaining a low fluoride level in oral fluids, thereby preventing caries.

Key words: Fluoride-containing pit-and-fissure sealant — Fluoride release — Fluoride recharge — Fluoride uptake — Caries prevention

INTRODUCTION

One of the key factors in preventing dental caries has been recognized to be increasing the resistance of teeth to acid by encouraging the development of re-mineralization mechanisms on the enamel surface. The daily topical application of a fluoride, such as sodium fluoride (NaF), acidulated phosphate fluoride solution (APF), or stannous fluoride (SnF_2), usually by the daily use of a fluoride-containing dentifrice, has been reported to be of benefit both in professional clinics and self care. Pit-and-fissure sealants are also useful

Table 1 Fluoride-containing pit-and-fissure sealants tested in this experiment

Trade name	Manufacturer/main components	Polymerization
Fuji III (FIII)	GC, Tokyo, Japan Powder: aluminum fluorosilicate glass, Liquid: polyacrylic acid and poly basic carboxyl acid	Chemical
Fuji III LC (FIII L)	GC, Tokyo, Japan Powder: aluminum fluorosilicate glass, Liquid: polyacrylic acid and 2-hydroxyethyl metacrylate (HEMA)	Light/Chemical curing
Teethmate F-1 (TF1)	Kuraray Medical, Osaka, Japan MDP, MF-MMA	Light curing
Helioseal F (HSF)	Vivadent, Liechtenstein Fluorosilicate glass	Light curing

in protecting against acid attack and can prevent caries in both primary and permanent molars.

It has been more than 30 years since Buonocore[3], and then Takeuchi and Kizu[15], developed plastic-based pit-and-fissure sealants in the 1960s. Since then, tremendous improvements and developments in new materials such as cyanoacrylate, bisphenol A-glycidyl methacrylate (Bis-GMA), methyl methacrylate (MMA), and polyurethane have been achieved and used clinically. Glass-ionomer-cement (GIC) based sealants were developed in the 1970s, and it was discovered that fluoride, which happened to be contained in these pit-and-fissure sealants, was slowly released into the oral cavity and contributed to both preventing dental caries and their recurrence in surrounding regions[2,12]. Therefore, intentionally adding fluoride to resin pit-and-fissure sealants through physical and chemical means has become widespread, not only to prevent dental caries but to seal the pits-and-fissures as well. However, clinical studies establishing the preventive effects of fluoride contained in sealing materials have not yet been published.

The purpose of this study was to evaluate the clinical usefulness of representative fluoride-containing pit-and-fissure sealants by examining their fluoride release during short periods and by testing their ability to recharge by measuring the fluoride uptake to the enamel surface adhering to the sealing materials *in vitro*.

MATERIALS AND METHODS

1. Fluoride-containing pit-and-fissure sealants materials

The pit-and-fissure sealants containing fluoride we investigated are listed in Table 1. They were Fuji III (FIII, GC Co., Tokyo, Japan), Fiji III LC (FIII L, GC Co., Tokyo, Japan), Teethmate F-1 (TF1, Kuraray Medical Co., Osaka, Japan), and Helioseal F (HSF, Vivadent Co., Liechtenstein). These had different hardening mechanisms and contained fluoride that was either there unintentionally or had been introduced intentionally.

2. Fluoride release from fluoride-containing sealant materials

The sealing materials were shaped into disks with an acrylic ring (11-mm outer diameter, 9-mm inner diameter, and 2.5-mm depth) according to the manufacturer's directions. The sealant material was placed into the acrylic ring lying on a piece of acrylic, and another piece of acrylic was placed on top. The acrylic pieces were then squeezed together to form a sealant disk. Only the FIII disks were left for a fixed time to allow chemical hardening the other materials were polymerized and hardened with light-irradiation (Jetlite 1000,

J. Morita Co., USA) from the top and bottom for 40 sec. After hardening, both the top and bottom acrylic were removed. Five disks were shaped from each sealant material. Each prepared disk was immersed in 5 ml of distilled water in a 10-ml plastic test tube and placed without agitation in an incubator at 37°C. The 5 ml of distilled water used for immersion was changed every 24 hrs.

3. Fluoride recharge of applied APF from sealants

The sealant disks were removed from the test tubes after immersion for seven days, rinsed with distilled water, and immersed in acidulated phosphate fluoride solution (APF: 9,050 ppmF, pH 3.8) in a 200-ml plastic beaker for 4 min. The disks were then removed from APF solution, re-rinsed with distilled water, and immersed in 5 ml of distilled water in a plastic test tube, enabling fluoride to be released. The immersing water was changed every day and this procedure was repeated over 14 days. The distilled water samples containing fluoride ions were kept in a refrigerator at 4°C until their fluoride concentrations could be determined.

4. Fluoride ion concentration measurements

The concentration of fluoride ions released from the sealants was analyzed with a combination fluoride ion-selective electrode (Model 96-09BN, Orion Research Co., Cambridge, MA) connected to an ion-analyzer (EA920, Orion Research Co., Cambridge, MA). To determine the fluoride concentration, 0.1, 1.0, and 10 ppm standard solutions of fluoride ions were prepared, and further standard solutions of 0.05 and 0.02 ppm fluoride ions were used for low fluoride solutions below 0.1 ppm each time.

5. Fluoride uptake to bovine enamel treated with FIII L and FIII L/APF application

Five bovine anterior teeth were polished with a hearthstone powder and a polishing brush for about one minute each and incised between the tooth crown and root with a diamond cutter. Each tooth crown was divided into four specimens to obtain a total of 20 bovine enamel specimens. Five bovine enamel specimens were randomly chosen, and filled with the pit-and-fissure sealant, FIII L (light/chemical curing), according to the manufacturer's directions. Two groups consisting of five such bovine enamel specimens were prepared. In the first group, fluoride uptake to the enamel was accomplished with FIII L alone in the second group, APF was applied seven days later. Five bovine enamel samples were evaluated as the control.

A bovine enamel specimen sealed with FIII L was immersed in 5 ml of 0.05 M monocalcium phosphate saturated solution with a pH of 7.4 adjusted with 5 M potassium hydroxide[10]. This was used as artificial saliva in a plastic test tube. The immersion solution was changed everyday. In the APF application group, bovine enamel specimens were immersed in the APF solution for four minutes seven days after immersion started, rinsed with distilled water, and re-immersed in the artificial saliva. In both groups, immersion in the artificial saliva was maintained over 14 days.

6. Bovine enamel specimen sampling after treatment with FIII L and FIII L/APF applications and determination of fluoride uptake

After the immersion experiments, the pit-and-fissure sealants were firmly yet carefully removed from the bovine enamel specimens of both groups with a spatula. After rinsing with distilled water, a window (5 × 5 mm) was made on the enamel surface of the specimens with dental wax, and the specimen was fixed to an acrylic rod (8-mm diameter, 11-cm long). The enamel was sampled with acid etching according to Ohkawa's method[13]. The enamel specimens were immersed in 0.4 ml of 0.5 M perchloric acid in a plastic test tube for 10 sec and then neutralized by adding 1.6 ml of 0.5 M sodium tricitrate. The same enamel specimens were continuously subjected to further acid treatment through immersion for 20 and then 30 sec. The fluoride concentrations in the sampling solutions

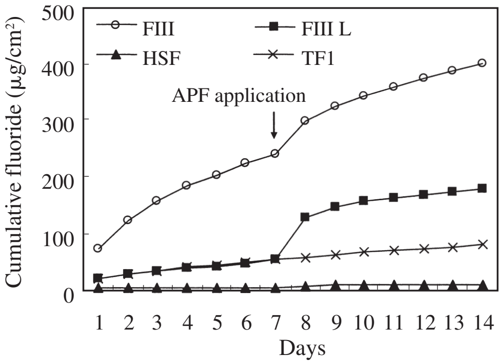

Fig. 1 Cumulative fluoride-release from four different pit-and-fissure sealants

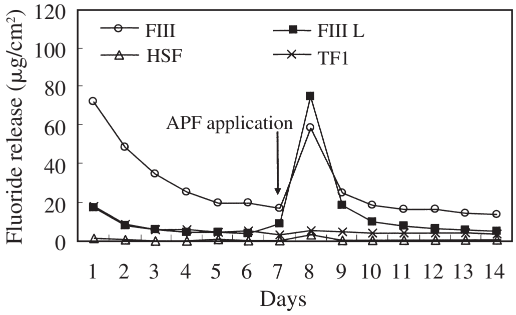

Fig. 2 Fluoride-release from each fluoride-containing pit-and-fissure sealant *in vitro*

were analyzed with the fluoride ion electrode method using a buffer solution, 0.5 M perchloric acid, and 0.5 M sodium tricitrate (1 : 4) as adopted by Ohkawa[13]. The calcium concentrations in the enamel sample solutions were measured with atomic absorption spectrometry (ASS: Model 508, Hitachi). The amount of enamel in each sample was calculated using a content of 36% calcium and 2.95 specific gravity for the values in bovine enamel. The area of the enamel specimen was determined with the tin foil method[13].

7. Statistical analysis

The means and standard deviations were calculated for the groups and evaluated by analysis of variance (Tukey Kramer test, StatView-J 5.0). A value of $p<0.05$ was regarded as significant.

RESULTS

Figure 1 plots the cumulative release of fluoride ions from the four types of fluoride-containing pit-and-fissure sealants. APF solution was applied on the seventh day. The cumulative release of fluoride ions up to the seventh day was the highest for FIII at 237 ± 45.2 (SD) $\mu g/cm^2$, FIII L and TF1 had similar levels of approximately 53 $\mu g/cm^2$, and HSF had the lowest value of $3.7 \pm 2.8\,\mu g/cm^2$. As we can see in Fig. 2, the rate at which fluoride ions were released from FIII was $71.9 \pm 12.6\,\mu g/cm^2$/day on the first day, and it rapidly decreased to $19.3 \pm 3.3\,\mu g/cm^2$/day on the fifth day. The rates at which fluoride ions were released from FIII L and TF1 had similar patterns. The initial fluoride release rates ranged from 17.2–18.4 $\mu g/cm^2$/day, and decreased to a third (4.0–4.7 $\mu g/cm^2$/day) of this on the fourth day, indicating that fluoride ions were released more slowly from FIII L and TF1 than from FIII.

We estimated the release of fluoride ions from the four sealants after APF had been applied on the seventh day and confirmed that fluoride was recharged in the FIII and FIII L GIC-sealants, although this was not observed in the TF1 or HSF resin sealants. Moreover, fluoride recharge was 1.5-fold greater in FIII L (light/chemical sealant $74.1 \pm 9.4\,\mu g/cm^2$) than in FIII (conventional sealant, $58.7 \pm 9.5\,\mu g/cm^2$), and the difference was statistically significant ($p<0.05$, see Fig. 3). Both instances of fluoride recharges were retained for about one day and it then took three days to reach the same release rate as that before APF had been applied.

Figure 4 plots fluoride concentrations in enamel layers in the GI, GII, and control groups. These concentrations for GI and GII were significantly higher than those of the control groups ($p<0.05$). The fluoride concentrations in GI were very high, i.e., $10{,}100 \pm 4{,}570$ (SD) ppm in the first layer, $6{,}070 \pm 3{,}430$ ppm in the second layer, and $3{,}350 \pm 1{,}840$ ppm in the third layer. The

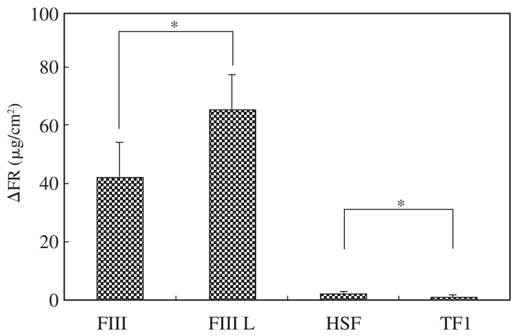

Fig. 3 Comparison of fluoride recharge (*Δ*FR, mean ± S.D.) after APF was applied for 4 min.

*Δ*FR is defined as the difference in fluoride release between that at 7 days and that at 8 days. *$p<0.05$

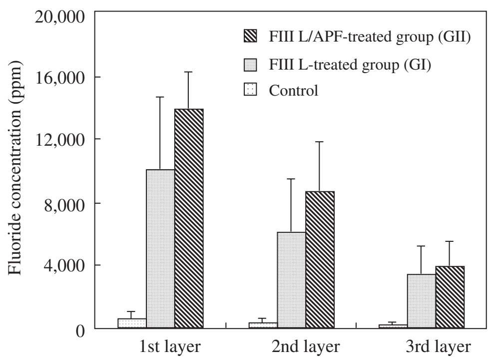

Fig. 4 Fluoride uptake (mean ± S.D.) to bovine enamel with Fuji III LC (GI) and Fuji III LC/APF (GII)

fluoride concentration in GII was about 13,900 ± 2,390 (SD) ppm in the first layer, 8,550 ± 3,260 ppm in the second layer, and 3,870 ± 1,620 ppm in the third layer. The mean fluoride uptake to the enamel surface was slightly higher in GII than GI, but there were no statistically significant differences.

DISCUSSION

The fluoride release rates and periods of fluoride-containing pit-and-fissure sealants are clinically important in establishing to what extent dental caries can be prevented and whether they will recur in the boundary. We evaluated the fluoride released from four types of fluoride-containing pit-and-fissure sealants in this study, fluoride recharge resulting from APF application, and fluoride uptake to enamel treated with FIII L/APF *in vitro*, to evaluate their clinical significance in preventing caries.

Since conventional GIC (FIII) is extremely sensitive to water, GIC that has been chemically polymerized after being used in fillings is detached if water and saliva contact it, suggesting that the rate at which fluoride ions are released is increased due to it detaching. As FIII L is light-hardening due to resin polymerization (HEMA), the degree to which it detaches is less than that of FIII, resulting in fluoride being released more slowly. This means that because FIII L is less sensitive to water it retains much more AFP/FIII.

We confirmed that fluoride is released from glass ionomer/resin pit-and-fissure sealants for long periods. We found a marked recharge after applying APF to both the conventional and autohardening GICs used for filling materials[11]. In the present study, the concentration of fluoride ions released from a light/chemical GIC sealant (FIII L) decreased to about a third immediately after APF was applied, which agreed with these results.

Similar results when examining short-term fluoride ion release and fluoride recharge (APF, NaF, SnF_2) in GIC materials have been reported, but the reported recharge of fluoride ions from the application solutions used in dental materials has been very low for NaF within the neutral pH range and it has been highly dependent on pH[4].

In the 1990s, Arends and Christoffersen[1] and Featherstone[6] proposed that dental caries could be prevented on superficial layers of enamel by inhibiting demineralization under common ions such as calcium ions and phosphate ions and by promoting remineralization under fluoride ions on the surface. They also stated that significant remineralization occurred even at very low concentrations of fluoride ions (0.03–0.05 ppm) in the oral cavity. Consequently, fluoride in dental materials that contributes to the release of fluoride ions is very important, both locally and throughout the entire oral cavity. However, in this study and many others, examining the release of fluoride ions from pit-and-fissure sealants and fluoride-containing materials has been done in batch systems with distilled water *in vitro*, resulting in limitations in evaluating the dynamics of fluoride ions emanating from these materials. Therefore, it is necessary to examine the dynamics of fluoride ions under conditions that are the same as the oral environment, for example, through studying pH cycling and the *in situ* oral system[5].

Ripa[14] suggested that there were limitations with intentionally adding fluoride to dental materials and devices to prevent dental caries from forming or enamel from demineralizing. These limitations extended to delivery of drugs (including fluoride) to prevent oral diseases (including those of soft and hard tissues) because the clinico-epidemiological findings were inconclusive[14].

The preventive effects the glass-ionomer pit-and-fissure sealant (FIII), were surveyed clinico-epidemiologically for three years by covering the permanent teeth of 91 children aged 4–10 years without caries with FIII[9]. They found that the DMFT value decreased to 66.5%. Furthermore, clinically reapplying glass-ionomer would be sufficiently simple for the public health sector, and its effects in preventing dental caries in children are undeniable.

We rejected the null hypothesis that there is little difference in fluoride recharge after APF treatment on the seventh day between sealants (Fig. 3), because we found the fluoride recharge in the FIII and FIII L GIC sealants to be much higher than that in the TF1 and HSF resin sealants ($p<0.05$). The fluoride recharge in FIII L was also significantly higher than that in FIII ($p<0.05$). Therefore, we confirmed that GIC sealants serve as fluoride reservoirs in this experiment. In relation to this, we also demonstrated that the acid-resistance in enamel treated with GIC sealant had been greatly improved[8].

We did not reject the null hypothesis that fluoride uptake to the enamel surface by FIII L was almost the same as that with FIII L/APF treatment on the seventh day, because there were no significant differences between the two fluoride concentrations. However, the fluoride concentration in each layer of enamel with FIII L/APF treatment (GII) tended to be slightly higher than that with FIII treatment (GI), although both these fluoride levels were extremely high. Regarding the fluoride uptake in enamel with resin sealants (MF-MMF co-polymerization polymer), Tanaka *et al.* reported that the concentration of total fluoride was ca. 3,500 ppm on a 10-μm superficial layer, that KOH-soluble fluoride (non-bound) was 20–30%, and that bound fluoride was 70–80% on layers up to 60 μm[16]. These results were significantly higher than those in the control group. Kawai *et al.* obtained the same results for another composite resin they investigated[7].

Our present study concurred with these previous results and found that fluoride-recharge from pit-and-fissure sealants and filling materials on the surface of teeth occurred within a short time and that their application to teeth increased acid resistance on the surface.

In the future, we should run clinico-epidemiological studies and *in vivo* experiments to evaluate the effects of fluoride-containing sealants on preventing caries.

CONCLUSIONS

Of the four types of pit-and-fissure sealants, conventional GIC (FIII) had the highest rate of fluoride release; a similar release pattern was observed in GIC pit-and-fissure sealant (FIII L) and resin sealant containing fluoride releasing polymer (TF1). The rate at which fluoride was released was reduced to about a third of the initial level within a few days, and release continued slowly thereafter. The fluoride recharge in FIII L when APF was applied to the GIC sealant was significantly higher than in FIII ($p<0.05$). These results suggest that GIC-sealants in the oral cavity reservoirs differ in their properties and that they can contribute to low levels of fluoride being retained in the oral fluid, thereby preventing caries.

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